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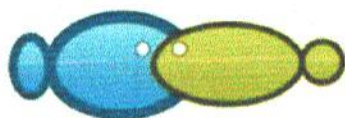
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## Effects of vermicompost on growth performance and antioxidant status of seaweed *Caulerpa racemosa*, South Sulawesi, Indonesia

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**Abstract.** Environmental factors have been known as the limiting factors in farming seaweed. The environmental factors more likely affect to the growth performance and quality content of seaweed. This study aimed to investigate the effects of organic vermicompost fertilizer on growth and antioxidant status of *Caulerpa racemosa*. Eighteen experiment land ponds with dimension 1 m x 1 m x 0.6 m and filled with brackish water with a height of 60 cm were applied. Growth performance, pigment and antioxidant content were measured. Two-way ANOVA was performed for statistical analysis. The results showed that the growth rate and biomass increased in all treatments (treatment A: 300 g m<sup>-2</sup>; treatment B: 250 g m<sup>-2</sup>; treatment C: 200 g m<sup>-2</sup>; treatment D: 150 g m<sup>-2</sup>; treatment E: 100 g m<sup>-2</sup>; treatment F: control without vermicompost). The highest mean value was found in the treatment B dose (250 g m<sup>-2</sup>). It suggested that the result of NH<sub>3</sub> uptake in the treatment doses of 250 (B) (32.55%) and 100 (E) (32.25%) increased respectively, whereas on day 28, the NH<sub>3</sub> uptake of seaweed in the same group of treatment decreased by 16.483% and 21.135% respectively. This nitrogen uptake promoted a growth of *C. racemosa* which given a vermicompost fertilizer. In addition, the highest value of antioxidant of *Caulerpa racemosa* was obtained from the dose of 250 g m<sup>-2</sup> (25.50%) while the lowest value was obtained in the dose of 150 g m<sup>-2</sup> (18%). In conclusion, the addition of vermicompost fertilizer as a source of nutrient in the tanks promoted growth and best antioxidant content. Nonetheless, the chlorophyll value showed no difference in all treatments.

**KeyWords:** *Caulerpa racemosa*, vermicompost, antioxidant, weight, chlorophyll.

**Introduction.** Seaweeds are the macro benthic and large primary producer in the marine water which has more than 300 species with economic value (Baleta & Nalleb 2016). *Caulerpa racemosa* (green algae) is one of the economic seaweed commodities which is known as sea grapes. Most green algae are found abundantly in the ocean surface, marine sediments, and inhabit in the sandy rock bottoms in sublittoral zone (Gennaro & Piazzini 2014; Asmida et al 2017). *C. racemosa* is locally known as "lawi-lawi", particularly in South Sulawesi. In the traditional market, *C. racemosa* is mostly obtained from wild yield. The local market for seaweed is still dominated by the *Kappaphycus alvarezii* and *Gracilaria* sp. Since *C. racemosa* has started to have higher market value along with other seaweed commodities such as *Eucheuma* sp. and *Gracilaria* sp., *C. racemosa* farming is required to decrease the dependency of its supply from the wild yield.

In order to meet the demand of high nutritional value and productivity of sea grapes, the technology for farming seaweed is required. Nonetheless, the quality of biochemical content of seaweed is highly affected by the environment. Naturally, seaweed store the nutrient from the environment into its tissue for promoting growth. This condition plays mutual influential for the growth and nutrient status of the seaweed.

The *C. racemosa* nutrient content can be improved through the addition of organic matter or fertilizer. Prior to rearing activity, the immersion treatment of the seaweed

seed often given using the organic fertilizer (Nurfebriani et al 2015). One of the great fertilizers that can be used to enrich the seaweed is vermicompost. In order to generate optimal results, it is necessary to understand the suitable soaking time for *C. racemosa* in different concentration of the vermicompost fertilizer. The reason for this study was to utilize more environmentally friendly and affordable aquaculture practices for the farmer community. In addition, vermicompost is very rich with various kinds of nutrients. Therefore, the aim of this study was to investigate the effect of vermicompost on growth and antioxidant status of *C. racemosa*.

**Material and Method.** This study was conducted in September 2018 using earthen ponds with several modifications. There were 18 experiment containers which were made by wooden tub coated with tarpaulin with 1 m x 1 m x 0.6 m and the base was covered with soil 10 cm thick, then filled with brackish water (pond water) with a height of 60 cm according to Kadi (2004).

**Experimental design.** Completely randomized design with six treatments and three replicates was applied for experimental design as follows:

- treatment A: 300 g m<sup>-2</sup> of vermicompost fertilizer;
- treatment B: 250 g m<sup>-2</sup> of vermicompost fertilizer;
- treatment C: 200 g m<sup>-2</sup> of vermicompost fertilizer;
- treatment D: 150 g m<sup>-2</sup> of vermicompost fertilizer;
- treatment E: 100 g m<sup>-2</sup> of vermicompost fertilizer;
- treatment F: control without vermicompost.

The initial weight of *C. racemosa* for all treatments was 100 g and reared for 28 days.

**Water quality measurements.** Water quality test kit API USA was used for the ammonia and nitrate test, whereas the total nitrogen (TN) test was colourimetrically determined using a Nessler method. All the water quality measurement were based on APHA (2005) recommendations.

**Growth parameters measurement.** The epiphytes were removed, and immediately rinsed with seawater. The seaweed was placed in aluminium trays for 5 minutes to reduce excess water. The initial weight and final weight of the seaweed were measured for growth performance. The samples were weighed using a Sartorius analytical balance (0.05 g). The specific growth rate (SGR) was determined according to Yang et al (2005) as calculated using the following equation:

$$\text{SGR (\% day}^{-1}\text{)} = ((\text{Ln } (W_t) - \text{Ln } (W_i))/t) \times 100$$

where: -Ln = the natural log;  $W_i$  = the initial weight (g);  $W_t$  = the final weight (g); and  $t$  = period in days.

**Pigments content analysis.** Chlorophyll-*a* is measured by extracting about 0.1 g of seaweed tissue in 5 mL of acetone 90% (v/v) using a homogenizer. Homogenate was disincubated at 2000 g for 15 minutes. Chlorophyll levels were determined by clumps described by Jeffrey & Humphrey (1975).

**Antioxidant analysis.** DPPH method was used for the antioxidant analysis (AOAC 2000) and the test for antioxidant activity and dietary fibre based on AOAC (AOAC 2011) was performed in the Laboratory of Animal Food Chemistry, Department of Nutrition and Animal Feed, Faculty of Animal Husbandry, Hasanuddin University.

**Data analysis.** Data experiments were statistically analyzed using IBM SPSS 22 for Windows. The results were expressed as the mean ± standard error (SE). Data were tested for homogeneity of group variance (Levene's test), and subjected to two-way ANOVA. Significant differences were further tested using Post-hoc tests at  $p$ -value < 0.05.

**Results and Discussion** The growth performance of *C. racemosa* which given different vermicompost fertilizer can be seen in Table 1.

Table 1

Specific growth rate and biomass

Fertilizer treatment ( $g\ m^{-2}$ )	Specific growth rate ( $\% day^{-1}$ )	Final weight (g)
A (300)	$1.64 \pm 0.96$	$461.67 \pm 1.27$
B (250)	$2.26 \pm 0.52$	$573.33 \pm 1.02$
C (200)	$1.43 \pm 0.35$	$356.33 \pm 1.20$
D (150)	$1.71 \pm 0.47$	$380.67 \pm 1.03$
E (100)	$1.03 \pm 0.16$	$275 \pm 1.65$
F (control)	$1.47 \pm 0.50$	$340.33 \pm 1.57$

From the Table 1 above, it can be seen that the growth rate and biomass in general experience an increase from the initial weight and this occurred in all treatments. The highest value was found in treatment B. Nonetheless, statistically, the vermicompost treatment dose did not give significant differences in term of growth rate. There was a difference between groups of treatment in the initial weight and at the end of the rearing period (Figure 1).

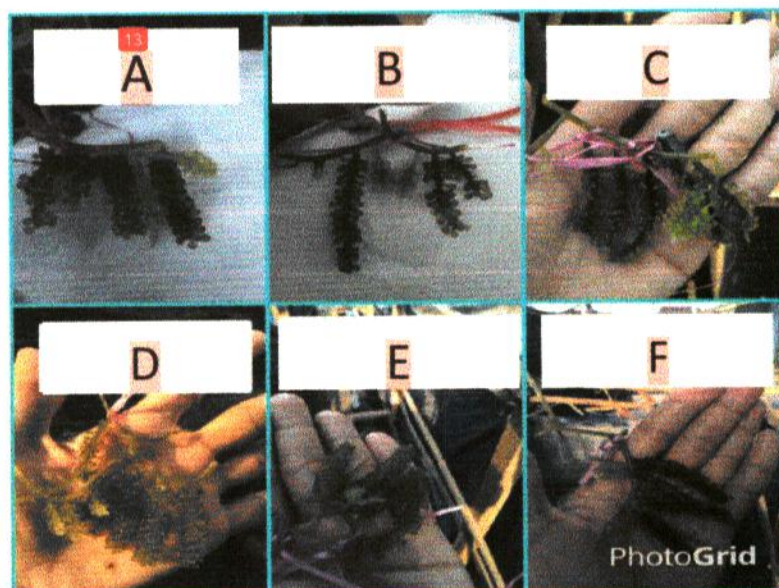


Figure 1. The growth of new thallus of *C. racemosa*. The above figures show the *Caulerpa racemosa* thallus obtained from each treatment group A-F = treatment groups).

Seaweed growth is indicated by the emergence of a new thallus. The growth of thallus really depends on the position of the seaweed in the water. According to Insan et al (2013) the different position (vertical or horizontal) of seaweed can cause the different acquisition of sunlight received by the thallus.

The effect of different fertilizer doses on the growth rate and biomass of *C. racemosa* certainly provides contradictive results with previous studies. Rahim (2017) claimed that the higher the dose given the higher the growth rate and biomass of *Gracilaria verrucosa*. It appears that *Caulerpa* species is not suitable with vermicompost fertilizer. Beside from the fertilizer, it is suspected that the *Caulerpa* seaweed does not

have a proper nutrient from the substrate so that the possibility of nutrient absorption by *Caulerpa* seaweed did not fully affect on growth and nutritional status of seaweed. This is might happen because of the lack of nutrient from the substrate that cannot be fulfilled by the vermicompost. This is relevant to Nurfebriani et al (2015) that *Caulerpa* sp. cannot grow normally in the rainy season because presumably due to lack of nutrient from the substrate that caused by decreasing of microorganisms. Microorganisms contained in the substrate play a role in the formation of carbon, nitrogen and other nutrients which are very necessary for plant growth.

Another possibility is thallus growth that occurs in *C. racemosa* was hampered by the growth of the old thallus so that there is competition between the thallus to get space in one cluster, especially in getting sunlight. According to Charrier et al (2017) and Melsasail et al (2018) that seaweed are low level macroscopic aquatic organisms that cannot be distinguished among the leaves, stems, and roots and also contain chlorophyll so being able to do photosynthesis. Through this process, seaweed absorbs CO<sub>2</sub> and H<sub>2</sub>O and nutrient complex molecules from the environment.

The Figure 2 illustrates that treatment on day 14 suggested a different pattern of nitrogen uptake with treatment on day 28 where the NH<sub>3</sub> percentage of seaweed in the treatment doses of 250 (B) (32.550%) and 100 (E) (32.252%) increased respectively, whereas on day 28, the NH<sub>3</sub> uptake of seaweed in the same group of treatment decreased 16.483% and 21.135% respectively. The high ammonia was recorded on day 14 because the application of fertilizer was only given at the beginning of the treatment, while vermicompost was a decomposer that is rich in nutrients, one of which is ammonia. The decline of nitrogen and phosphate source occurred on the day due to the *C. racemosa* actively utilized the nitrogen for growth. This is indicated by the growth rate of the seaweed sample during the study period. This was confirmed by the control treatment (without fertilizer) where the ammonia value decreased both on day 14 to day 28. This is supported by Rahim (2017) that vermicompost is rich in nutrients, especially phosphate elements.

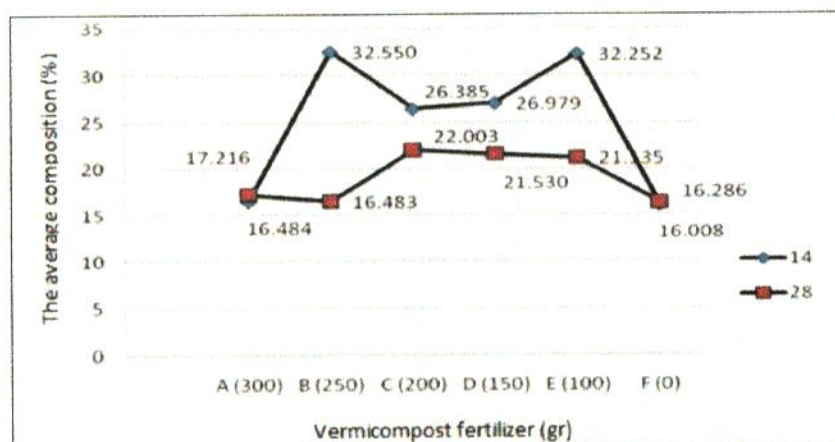


Figure 2. Nitrogen uptake (NH<sub>3</sub>) of *C. racemosa* with different vermicompost concentration.

Based on Table 2, the highest value of antioxidant of *C. racemosa* was obtained from the B (25.50%) while the lowest value was obtained in the dose of D (18%). This antioxidant value is obtained by testing the same sample concentration of 200 g and calculated after 28 days of rearing. The activity value is expressed in percent inhibition (% I) and as a standard used ascorbic acid (vitamin C). The parameters measurement was the antioxidant activity (IC<sub>50</sub>) of *C. racemosa* which suggests a value that shows the concentration of extract which is able to inhibit the activity of a radical by 50% which means that the smaller IC<sub>50</sub> value indicates higher antioxidant activity (Pyrzynska & Pękal 2013). In other words, the overall value of antioxidant in the present study suggested

the best antioxidant value because all treatment was below 50%. This is in accordance with Suratmo (2010) that the value of the level of antioxidant strength with the DPPH method if it is below 50 % is included in the very strong category. The table of antioxidant values can be seen in Table 3.

The antioxidant value of *Caulerpa racemosa*

Table 2

Days	Antioxidant values (%)					
	A (300)	B (250)	C (200)	D (150)	E (100)	F (control)
0	27.61	27.61	27.61	27.61	27.61	27.61
28	21.88	25.5	24.03	18	21.48	21.4

The level of antioxidant intensity using the DPPH method (Suratmo 2010)

Table 3

The intensity of antioxidant	Value of $IC_{50}$
Very strong	< 50 ppm
Strong	50-100 ppm
Moderate	100-250 ppm
Weak	250-500 ppm

The best result suggested by the concentration of D with a value of 18 % for 28 days of rearing period. This is in accordance with the statement of Djapiala et al (2013) that *C. racemosa* is known to contain phenolic compounds, which act as antioxidants.

Some chlorophyll-*a* content experienced a decline on day 14. However, there was a slight increase from the initial value of chlorophyll-*a* on day 28. The highest average value of chlorophyll-*a* was at a dose of 100 g m<sup>-2</sup> but it's slight different only with other doses (Figure 3). Statistically, the results of the present study at an early period of rearing showed an effect on the value of chlorophyll-*a* ( $p < 0.05$ ) but at the end of the study with a value of  $p > 0.05$  showed that there was no difference in chlorophyll-*a* value due to the addition of fertilizer doses. The absence of the effect of adding this dose is probably due to less exposure of sunlight to the water column of rearing pond. As a result, the value of chlorophyll obtained from all treatments tends to be similar even though the dosage given is different. This means that probably the amount of light level received by the seaweed is all at the same level. This is in accordance with Vogelmann & Gorton (2014) that balancing act between light level and carbon dioxide supply are two limiting factors for the photosynthesis process.

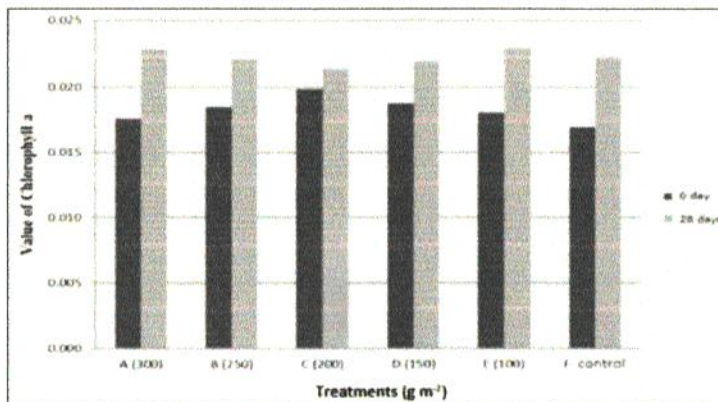


Figure 3. The average increase of chlorophyll-*a* value in all treatments from the beginning to the end of the rearing period.

**Conclusions.** The addition of vermicompost fertilizer as a source of nutrient in the ponds promotes growth. The highest growth rate was found in treatment B. Nonetheless, statistically, the vermicompost treatment did not give significant differences in term of growth rate between treatments. Although the statistical result shows no difference in all treatment, the highest value of antioxidant of *C. racemosa* was obtained from the B treatment. In addition, the chlorophyll-*a* value showed no difference in all treatments.

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